Hypertonic Saline Resuscitation Modulates Neutrophil Adhesion Molecule Expression of Post-Traumatic Hemorrhagic Shock Patients

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SUMMARY

Context - Fluid resuscitation for traumatic hemorrhagic shock remains controversial since current protocols using large-volume crystalloid may exacerbate post-traumatic inflammation and organ dysfunction. Experimental data suggests that hypertonic saline/dextran (HSD, 7.5%NaCl in 6%dextran-70) exerts anti-inflammatory and immunomodulatory effects, reduces multiorgan dysfunction and improves outcome. Objective – First trial examining the immunomodulatory properties of HSD in humans. Based on experimental findings, this study proposes to determine whether a single bolus of HSD might modulate the inflammatory response to hemorrhagic shock. Design - Randomized, double-blinded, placebo-controlled clinical trial. Setting - Largest tertiary-care university-affiliated Trauma Centre in Canada. Patients - 27 adult patients, victims of blunt trauma and hypotension due to hemorrhage. 13 patients received HSD, 2 excluded for failing inclusion criteria and 1 for refusing participation. Intervention – Upon arrival, eligible patients received a single 250-ml bolus of either HSD or placebo (0.9%NaCl) from unmarked bags. Blood samples were collected prior to infusion and over subsequent 24 hours. All patients underwent standard resuscitation. Main Outcomes - HSD markedly altered shock-induced changes in key adhesion molecules on circulating neutrophils compared to placebo. HSD abolished shock-induced upregulation of CD11b and caused extensive CD62L shedding. Leukocyte counts were similar, except for lymphocytes where HSD prevented the lymphopenia detected in the control group. Other Results – HSD patients required less crystalloid and blood during the first 24 hours. HSD patients were liberated from mechanical ventilation 24 hours earlier than control. ICU stay, organ dysfunction, infections and mortality did not differ between groups. HSD modestly increased serum sodium and osmolarity. No complications were associated to HSD. Conclusions – A single bolus of HSD during early resuscitation alters shock-induced inflammatory response by blunting neutrophil activation and preventing post-shock lymphopenia. These findings are consistent with experimental data and provide "proof of principle" for larger clinical trials.

1.0 INTRODUCTION

Hemorrhagic shock and ensuing inflammatory response are prime contributors to morbidity and mortality in trauma.(1-3) While current resuscitation protocols using large-volume crystalloids can correct the shock state, they simultaneously appear to exacerbate systemic inflammation and the development of organ injury.(4-6) Specifically, abnormal activation of polymorphonuclear neutrophils (PMNs) and altered PMN-endothelial interactions have pivotal roles in post-resuscitation multiorgan dysfunction (MOD).(7)

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Both human and experimental animal studies demonstrated that a single small-volume (4 ml/kg) infusion of hypertonic saline plus dextran (HSD, 7.5%NaCl 6%dextran-70) effectively restores hemodynamics by osmotically driving extravascular fluid into the vasculature, immediately correcting systemic ischemia and lessening organ edema.[8;9] Early randomized controlled trials (RCT) proved that HSD is effective and safe [10-12], but lacked power to demonstrate more than trends toward better patient outcome.

Recent experimental data demonstrating the anti-inflammatory and immunomodulatory properties of hyperosmolar solutions, particularly with respect to neutrophils, renewed the interest in HSD.[4;13;14] *In vivo* experimental models, hypertonicity prevented PMN activation, adhesion and transmigration into tissues by altering surface expression of key adhesion molecules (CD11b and CD62L), thus attenuating inflammation and MOD.[13;15-19] Hypertonicity also enhanced lymphocyte proliferation, reverting post-traumatic immunosuppresion.[15;16;20;21] All these effects remain unsubstantiated in human trauma patients.

This is the first RCT investigating the immunomodulatory properties of HSD in hemorrhagic-shock patients. Consistent with experimental observations, HSD alters shock-induced inflammation by blunting neutrophil activation and preventing post-shock lymphopenia, providing "proof of principle" for larger clinical trials.

2.0 METHODS

2.1 Study Population

This prospective, randomized, double-blinded, placebo-controlled trial of HSD resuscitation for traumatic hemorrhagic shock was conducted at Sunnybrook Women's College Health Sciences Centre between April 2001–August 2002 (**Figure 1**). Institutional Review Board approved the study with provisions for delayed informed consent obtained within 24 h.

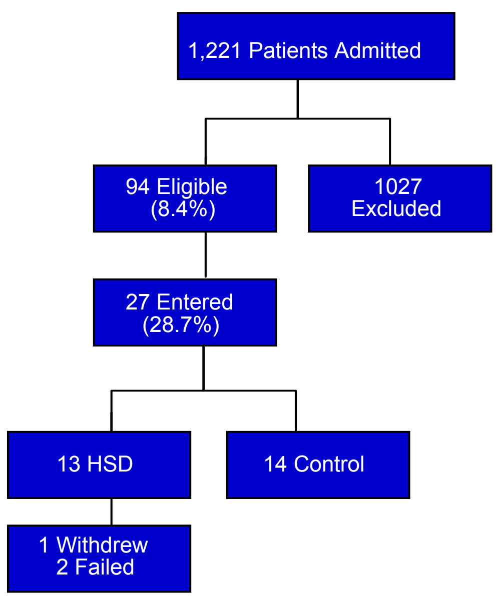

Figure 1. Number of patients assessed and enrolled in the trial.

Table 1 displays inclusion/exclusion criteria. Upon arrival, eligible patients randomly received a single 250-mL intravenous bolus of either HSD (7.5%NaCl 6%dextran-70) or placebo (0.9% NaCl) from identical unidentified bags. Resuscitation otherwise adhered to ATLS® guidelines. Patients were followed until hospital discharge or death.

Table 1: Inclusion and exclusion criteria according to the study protocol.

Inclusion (all of the following)	Exclusion (any of the following)
adults (16 years or older)	refusal to participate
blunt trauma	more than 6 hours after trauma
at least 1 episode of hypotension (systolic BP = 90 mmHg)	vital signs absent or not expected to survive 24 hours
evidence of blood loss (external, thorax, abdomen, retroperitoneum)	pregnancy or stigmata of chronic disease

2.2 Laboratory Procedures

Blood samples were collected before infusion, 1, 3, 6 and 24h after. As described(13), 100μL aliquots of unstimulated and endotoxin-stimulated (1μg/ml *Escherichia coli* 055:B5, Sigma) whole-blood were stained with anti-CD11b-FITC and anti-CD62L-PE monoclonal antibodies and analyzed by flowcytometry (BD). Soluble CD62L was measured by sandwich ELISA (Bender MED-Systems).

2.3 Outcome Measures

Primary outcome measure was change in neutrophil activation as measured by surface expression of adhesion molecules (CD11b and CD62L). Secondary outcomes included changes in leukocyte counts, serum sodium/osmolarity, and 24h fluid/blood requirements. Additional data collected prospectively included serum chloride, potassium, creatinine, lactate, bicarbonate, PT/PTT, ICU/hospital stay, mechanical ventilation, mortality, organ dysfunction measured by changes in multiorgan dysfunction score (ΔMOD) (ICU admission minus mean daily ICU scores)(22) and pneumonia (fever, leukocytosis, radiological infiltrate, sputum culture).

2.4 Statistical Analysis

Treatment comparisons for continuous variables used repeated measures ANOVA and Pearson χ^2 or Fischer's Exact test for categorical variables. Data are expressed as means ± standard deviation (SD) with a .05 significance level.

3.0 RESULTS

3.1 Study Population

During study period, among 1,121 trauma patients admitted, 94 (8.4%) fulfilled inclusion criteria and 27 enrolled with 13 receiving HSD and 14 placebo. Three HSD patients were excluded, 2 lacking required hemorrhage and 1 refusing participation (**Figure 1**). Both groups had similar characteristics and pre-admission interventions (**Table 2**). Patients randomized to HSD received more crystalloid before enrolment (p=.04), reflecting longer pre-hospital time and greater likelihood of referrals from other hospitals (ns, not significant).

No significant differences in clinical outcome were detected. However, HSD patients required less crystalloid in the Emergency Room (ns) and half the amount of blood (ns) and colloids (p=0.02) during initial 24h. All patients required ICU admission with similar length of stay. HSD patients were liberated from mechanical ventilation 24h earlier than control (ns). MOD and pneumonia affected both groups equally. Two control patients died, shortening the group's hospital stay (**Table 2**).

Table 2 – Baseline characteristics and outcomes of the study patients.

	Control (n=14)	HSD (n=10)	*p* value	
Age, mean (SD), years	47.5 (15.9)	49.3 (16.7)	.75	
Gender, male, no. (%)	9 (64%)	7 (70%)	.76	
ISS, mean (SD)	25.9 (10.3)	26.3 (11.4)	.83	
Mechanism injury – MVA, no. (%)	9 (65%)	8 (80%)		
Fall, no. (%)	1 (7%)	2 (20%)		
Other, no. (%)	4 (28%)	0 (0%)		
Transferred from other institution, no. (%)	5 (36%)	7 (70%)	.09	
Time - pre-hospital, mean (SD), min	110.5 (66.9)	172.4 (82.9)	.49	
in Emergency Room, mean (SD), min	114.6 (53.5)	164.5 (95.7)	.24	
Lowest systolic BP in ER, mean (SD)	90 (22.7)	80 (15.6)	.31	
Highest HR in ER, mean (SD)	114 (17.1)	110 (12.6)	.29	
Crystalloid - pre-hospital, mean (SD), ml	835 (855)	2144 (1343)	.048	#
ER, mean (SD), ml	4542 (2758)	3689 (1865)	.28	
total first 24h, mean (SD), ml	8080 (2736)	7796 (3189)	.75	
Blood - pre-hospital, mean (SD), units	0.5 (1.16)	1.22 (1.7)	.27	
ER, mean (SD), units	1.56	1.5	.62	
total first 24h, mean (SD), units	4.36 (6.77)	2.2 (2.9)	.38	
Colloids - total first 24h, mean (SD), ml	696 (773)	361 (377)	.02	#
LOS - total hospital stay, mean (SD)	27.4 (11.7)	36.9 (43.7)	.048	#
- ICU stay, mean (SD)	8 (8.2)	7.9 (6.8)	.3	
Patients operated first 24h, no. (%)	10 (71.4%)	6 (60%)	.55	
Number surgical procedures/patient, mean (SD	2.6 (2)	2.2 (2)	.21	
Complications – vent time, mean (SD), days	5.3 (6.2)	4.3 (7.2)	.91	
pneumonia, number patients	1.43 (.51)	1.3 (.48)	.22	
∆ MOD score, mean (SD)	1.9 (4)	1.68 (2.4)	.16	
Death	2 (14.3)	0	.21	

SD = standard deviation, no. = number, ISS = injury severity score, MVA = motor vehicle accident, min = minutes, ml = milliliters, ER = emergency room, ICU = intensive care unit, vent = mechanical ventilation, ∆MOD = delta multiple organ dysfunction (Marshall et al, Crit Care Med 1995; 23(10):1638-1652), # = statistically significant.

3.2 Primary Outcome

Hemorrhage/resuscitation caused a marked and progressive in CD11b expression up to 24h (**Figure 2**). HSD inhibited shock-induced CD11b upregulation, sustaining it below baseline for the entire 24h period.

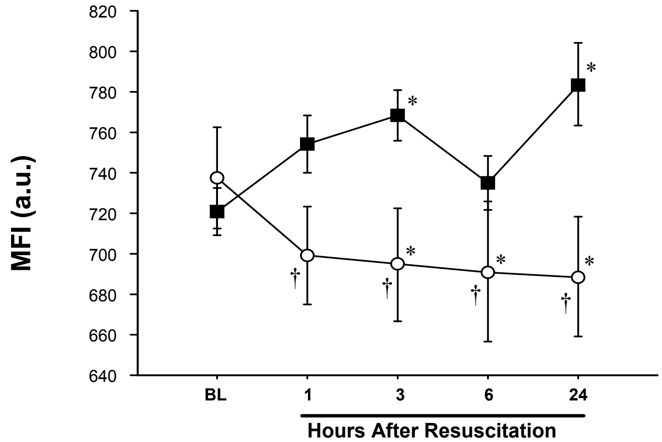

Figure 2. Neutrophil CD11b surface expression. Neutrophils were isolated from patients receiving a single 250 mL dose of HSD (7.5% NaCl 6%dextran-70,) or placebo (0.9% NaCl,), labelled with anti-CD11b-FITC monoclonal antibody and analysed by flow cytometry. Y-axis measures mean fluorescence intensity (MFI) in arbitrary units (a.u.). BL = baseline (prior to infusion); *significantly different from BL; †significantly different from control group.

Hemorrhage/resuscitation per se caused no change in PMN CD62L expression (**Figure 3**). By contrast, HSD caused progressive reduction in CD62L expression, reaching a nadir at t=3h and remaining low over the entire experimental period.

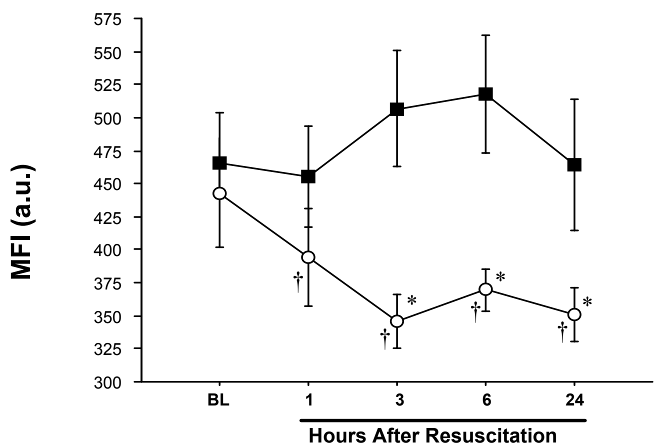

Figure 3: Neutrophil CD62L surface expression. Neutrophils were isolated from patients receiving a single 250 mL dose of HSD (7.5% NaCl 6%dextran-70,) or placebo (0.9% NaCl,), labelled with anti-CD62L-PE monoclonal antibody and analysed by flow cytometry. Y-axis measures mean fluorescence intensity (MFI) in arbitrary units (a.u.). BL = baseline; *significantly different from BL; †significantly different from control.

Endotoxin stimulation elicited expected changes in both CD11b/CD62L from all patients, attesting PMN viability/functionality (**Figure 4A,B**). The reduction of CD62L surface expression paralleled an increase in serum soluble CD62L (**Figure 5**), suggesting activation-induced shedding mechanisms.

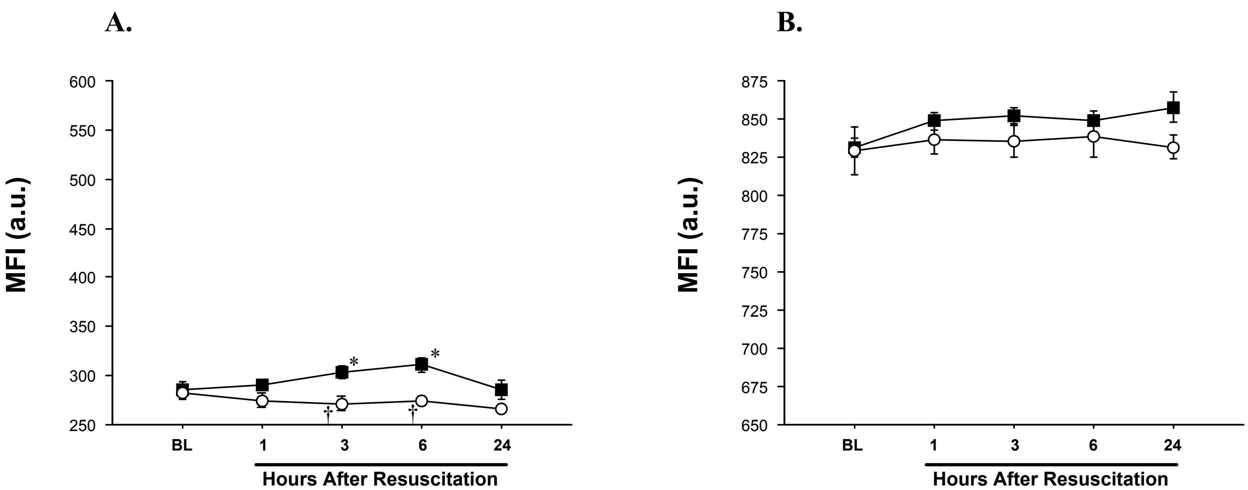

Figure 4: Neutrophil surface expression of CD11b (A) and CD62L (B) after endotoxin (LPS) stimulation. A, B – to attest viability and functionality, neutrophils from HSD (–○–) and placebo (–■–) groups were stimulated with 1μg/ml *E. coli* 055:B5 for 1 hour, labelled with specific monoclonal antibodies then analysed by flow cytometry. Y-axis measures mean fluorescence intensity in arbitrary units. BL = baseline, *significantly different from BL, †|significantly different from control group.

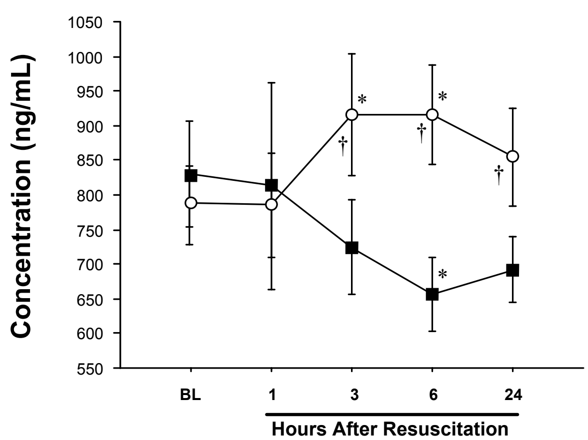

Figure 5: Serum soluble CD62L was measured to confirm that the reduction in CD62L surface expression was due to activation-induced shedding, by ELISA. Y-axis measures serum concentration (ng/mL) in HSD (–○–) and placebo (–■–) groups. BL = baseline, *significantly different from BL, †|significantly different from control group.

3.3 Secondary Outcomes

All patients experienced a drop in total leukocyte and neutrophil counts. HSD abolished the marked lymphopenia observed in the control group, without affecting other counts (**Table 3**). HSD modestly increased serum sodium/osmolarity, with normalization by 24 h (**Table 4**). No differences in serum chloride, potassium, creatinine, lactate, bicarbonate and PT/PTT were detected. HSD did not affect blood typing/crossmatching.

Table 3: Total leukocyte, granulocyte and lymphocyte counts at baseline and after resuscitation.

Counts ($x10^9/L$)	Sample Time					***P* Value compared to Baseline**
	BL	1h	3h	6h	24h	
Leukocytes						*P* < .01
Control	14.75 ± 0.92	14.41 ± 1.82	12.51 ± 1.56*	10.87 ± 1.07*	11.08 ± 0.84*	
HSD	13.09 ± 0.96	14.41 ± 1.82	12.26 ± 0.89*	10.83 ± 0.67*	9.55 ± 0.75*	
Granulocytes						*P* < .01
Control	12.01 ± 0.82	12.18 ± 1.50	10.15 ± 1.34	8.81 ± 0.89*	8.46 ± 0.83*	
HSD	10.92 ± 0.80	10.59 ± 0.92	10.30 ± 0.80	9.12 ± 0.65*	7.74 ± 0.69*	
Lymphocytes						*P* < .02
Control	1.75 ± 0.26	1.37 ± 0.29	0.77 ± 0.13*	0.72 ± 0.10*	1.35 ± 0.22	
HSD	1.41 ± 0.24	1.12 ± 0.14	1.25 ± 0.22	1.16 ± 0.17	1.07 ± 0.14	

BL = baseline (prior to infusion); *significantly different from baseline.

Table 4: Changes in serum sodium and osmolarity at baseline and after resuscitation.

	Sample Time				
	BL	1h	3h	6h	24h
Sodium (nmol/L)					
Control	141.0 ± 3.5	139.2 ± 5.7	139.0 ± 4.6	140.2 ± 4.9	140.0 ± 4.2
HSD	141.1 ± 3.4	146.7 ± 2.8*†	145.5 ± 4.3*†	146.1 ± 3.8*†	144.0± 3.9
Osmolarity, (mosmol/kg)					
Control	297.6 ± 8.1	299.0 ± 2.8	299.3 ± 4.2	288.5 ± 5.7	288.7 ± 1.2
HSD	298.3 ± 7.8	304.1 ± 11.3	310.5 ± 2.1*†	309.0 ± 1.4*†	289.8 ± 5.1

BL = baseline (prior to infusion), *significantly different from baseline, †significantly different from control.

4.0 DISCUSSION

Neutrophil sequestration in various target organs following shock/resuscitation contributes to the pathogenesis of multiorgan dysfunction .[23] Two adhesion molecules, CD11b and CD62L are central to the events leading to PMN-endothelial adhesion with resultant endothelial damage, increased capillary leak and PMN transmigration with oxidative/proteolytic organ damage.[7;23-25] The present double-blinded RCT investigated the effect of a single dose of HSD on one critical parameter of PMN activation, the surface expression of adhesion molecules CD11b and CD62L, in patients undergoing hemorrhagic shock resuscitation. HSD caused progressive CD62L shedding as evidenced by reduced surface expression and the presence of soluble CD62L in the blood. Furthermore, HSD prevented shock/resuscitation-induced rise in surface CD11b. These effects mirror recent experimental data and are comparable to reports from normal volunteers.[26] While these effects did not translate in beneficial outcomes, the study was clearly not designed to test this possibility but rather to establish the principle that this intervention might prove salutary in larger patient trials. Our results further corroborate earlier trials demonstrating that HSD represents a safe additive to resuscitation.[10-12]

The anti-inflammatory effects on PMN we observed are consistent with studies suggesting that HSD has organ protective properties.[8;10;27] In a study by Simmas, no children with post-traumatic intracranial hypertension treated with HS developed ARDS versus 30% following conventional treatment.[27] Wade *et al* demonstrated that survival was twice as high for hypotensive severe head injury patients resuscitated with HSD.[28] Mattox reported less pneumonia [10], while the HSD-treated patients in this trial required shorter ventilatory support.

Besides attenuating inflammatory damage, HSD also reduces fluid requirements, immediately restores organ perfusion and reduces post-shock organ edema, all factors that might lessen MOD.[9;13;17] In contrast, standard large-volume resuscitation may increase brain edema, prolong mechanical ventilation and worsen outcome.[6]

In experimental models, hypertonicity inhibits PMN activation in both animal and isolated human cell preparations, while return to isotonicity restores competency.[4;13;18;29] We found that HSD exerted prolonged effects on PMN CD11b/CD62L expression (24 h), even after normalization of serum osmolarity. Possible explanations include osmotic effects on different cells and tissues, whereby interactions with PMNs, prolong HSD anti-inflammatory properties. Another possibility, compatible with a prolonged effect, is that HSD reduces the initial inflammatory outburst, such that even after the amplification cascade, the overall result is of an attenuated inflammation. Furthermore, attenuated initial inflammation generates less anti-inflammatory compensatory mechanisms, potentially reducing late immunosuppression. Both reduced initial systemic inflammatory response and reversal of late immunosuppression are well described effects of HSD in experimental models.[16;30;31]

Trauma/hemorrhage suppresses lymphocyte proliferation and function, resulting in immunosuppression, sepsis and MOD.[16;21;32] In experimental models HSD rescues T-lymphocytes, restoring their proliferative capability [16;21], decreasing sepsis/MOD. [30;32] We found that HSD reduced post-traumatic lymphopenia, suggesting similar effects on human lymphocytes.

5.0 CONCLUSION

This trial substantiates experimental observations on the effects of osmolarity in neutrophils and lymphocytes, translating basic science investigations into the clinical situation. It demonstrates for the first time the immunomodulatory effects of HSD in humans. The potential association of such effects with reduced organ dysfunction and better outcome provides proof of principle for further investigations. HSD may in fact prove to be an effective immunomodulatory agent in the broader range of conditions caused by ischemia/reperfusion injury.

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